

Identification of Two New HLA-A*1101-Restricted Tax Epitopes Recognized by Cytotoxic T Lymphocytes in an Adult T-Cell Leukemia Patient after Hematopoietic Stem Cell Transplantation

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We previously reported that Tax-specific CD8⁺ cytotoxic T lymphocytes (CTLs), directed to single epitopes restricted by HLA-A2 or A24, expanded in vitro and in vivo in peripheral blood mononuclear cells (PBMC) from some adult T-cell leukemia (ATL) patients after but not before allogeneic hematopoietic stem cell transplantation (HSCT). Here, we demonstrated similar Tax-specific CTL expansion in PBMC from another post-HSCT ATL patient without HLA-A2 or A24, whose CTLs equally recognized two newly identified epitopes, Tax88-96 and Tax272-280, restricted by HLA-A11, suggesting that these immunodominant Tax epitopes are present in the ATL patient in vivo.

Adult T-cell leukemia (ATL) caused by human T-cell leukemia virus type I (HTLV-I) is characterized by poor prognosis following chemotherapy (7, 18, 20, 23). However, the results of recent allogeneic hematopoietic stem cell transplantation (HSCT) for ATL patients are encouraging (9, 24). This indicates that a graft-versus-leukemia (GVL) response may be effective for ATL as well as other types of leukemia, although there is a risk of graft-versus-host (GVH) diseases (GVHD).

We previously found that peripheral blood mononuclear cells (PBMC) from ATL patients after but not before HSCT from HLA-identical donors exhibited vigorous HTLV-I-specific cytotoxic T lymphocyte (CTL) responses that were directed to a limited number of Tax epitopes, i.e., an HLA-A2-restricted Tax11-19 epitope in one patient and an HLA-A24-restricted Tax301-309 epitope in another (6). These patients have now been in complete remission for more than 3 years.

Since HTLV-I Tax is the dominant target antigen recognized by HTLV-I-specific CTLs (8, 10, 16), which are thought to be responsible for in vivo immune surveillance for HTLV-I leukemogenesis (11), the positive conversion of Tax-specific CTL responses in post-HSCT ATL patients suggested that these CTLs might be involved in a GVL response. In a rat model of HTLV-I-infected T-cell lymphomas, Tax oligopeptide at a dominant CTL epitope successfully induced antitumor immunity, implying that the dominant CTL epitope identified in ATL patients may also be a potential candidate for a tumor vaccine (5, 15).

In the present study, we analyzed T-cell responses in another

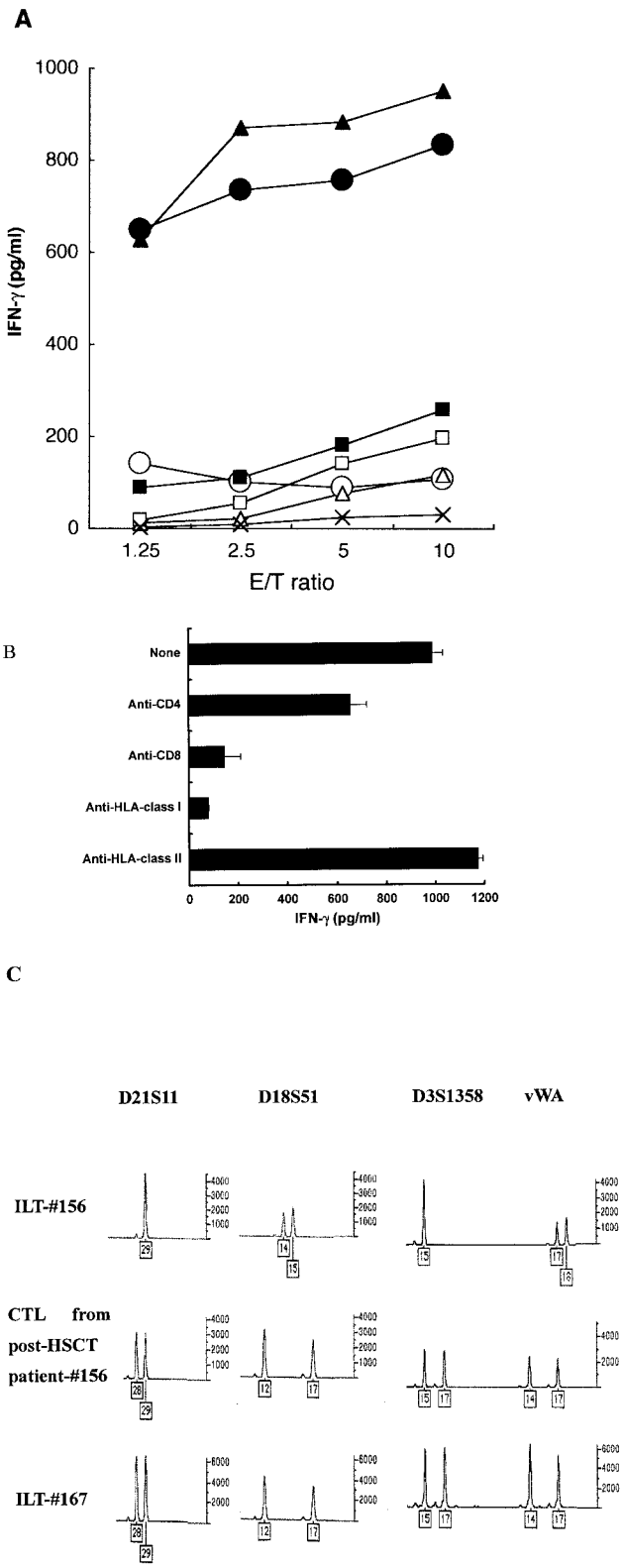
post-HSCT ATL patient without HLA-A2 or A24 and identified two new HLA-A11-restricted epitopes.

PBMC from an acute-type ATL patient (patient 156, a 51-year-old male) at 145 days after HSCT and from his HLA-identical (HLA-A11/A26, B52/B61 DR6/DR15) sibling donor (donor 167, a 55-year-old male) were collected after signed informed consent. Patient 156 obtained complete remission within 2 months after HSCT and sustained remission for longer than 15 months, although chronic GVHD was observed from 8 months after HSCT. Donor 167 was negative for HTLV-I.

A spontaneously HTLV-I-infected T-cell line (ILT-156) established from the PBMC of patient 156 before HSCT and an exogenously HTLV-I-infected T-cell line (ILT-167) established from PBMC of the seronegative donor 167 were maintained in the presence of interleukin-15. An Epstein-Barr virus-transformed lymphoblastoid cell line (LCL-156) was established from PBMC of patient 156 before HSCT, as described elsewhere (6, 22).

CD8⁺ PBMC isolated from post-HSCT patient 156 at 147 days after HSCT were cocultured with 1% formalin-treated ILT-156 cells, derived from pre-HSCT patient 156, twice with a 14-day interval in the presence of interleukin-2. The responder PBMC vigorously proliferating in culture at 17 days after initiation of culture produced significant levels of gamma interferon (IFN- γ) against ILT-156, but not against LCL-156, following overnight incubation (Fig. 1A). Cytotoxicities of the CTLs against ILT-156 cells were confirmed by ⁵¹Cr release assay. Significant levels of IFN- γ response were observed against allogeneic HTLV-I-infected cells sharing only HLA-A11 (TCL-Kan) but not against the ones sharing only HLA-A26 (ILT-Nkz-2). IFN- γ production of the responder cells against ILT-156 cells was significantly inhibited by treatment of responder cells with anti-CD8 monoclonal antibody (MAb) or

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by treatment of target cells with anti-HLA-class I MAb (Fig. 1B), confirming that the responder cells induced from post-HSCT patient 156 contained CD8-positive, HLA-A11-restricted, HTLV-I-specific CTLs.

The hematopoietic system in post-HSCT patient 156 when

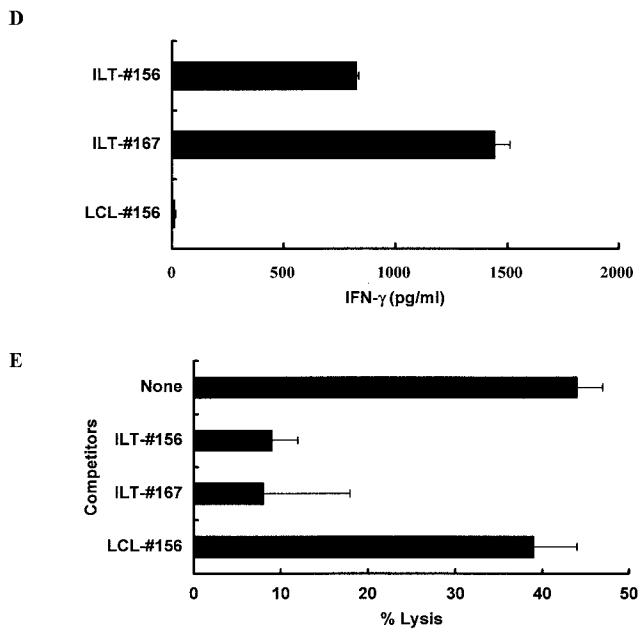


FIG. 1. Induction of CTLs in PBMCs from post-HSCT patient 156 by stimulation with ILT-156 cells. (A) PBMCs from patient 156 (147 days post-HSCT) were cultured with periodic stimulation with formalin-fixed ILT-156 cells, and their IFN- γ -producing ability was evaluated by enzyme-linked immunosorbent assay at 17 days after initiation of culture, following 18 h of incubation with ILT-156 (closed circles), LCL-156 (open circles), HLA-A11-matched TCL-Kan (closed triangles) (HLA-A2/A11, B7/Bw46, Cw1/Cw3/Cw7, and DR2/DR9) and LCL-Kan (open triangles) (same HLA type as TCL-Kan), HLA-A26-matched ILT-Nkz-2 (closed squares) (HLA-A2/A26, B51/B54, and Cw1/-) and LCL-Nkz (open squares) (same HLA type as ILT-Nkz-2), or no addition (crosses) at an effector cell/target cell ratio of 10. Closed symbols, HTLV-I-positive cells; open symbols, HTLV-I-negative cells. (B) The IFN- γ -producing ability of the CTLs induced from patient 156 at 25 days in culture was determined after 18 h of incubation with ILT-156 cells at an effector cell/target cell ratio of 10, following preincubation of effector cells with CD4 or CD8 MAbs or preincubation of target cells with HLA class I or class II MAbs for 1 h at 37°C (3). (C) STR polymorphism in DNA extracted from CTLs from post-HSCT patient 156, ILT-156 cells, and ILT-167 cells was analyzed by using an AmpFISTER SGM Plus PCR Amplification Kit, GeneScan 3.1, and Genotyper 2.5 software (Applied Biosystems, Foster City, CA). Electropherograms of four representative STR loci (D21S11, D18S51, D3S1358, and vMA) are shown. Peak height is measured against an arbitrary scale displayed on the y axis. The numbers of STRs are indicated in squares on the x axis. (D) IFN- γ production by CTLs from patient 156 (at 41 days of culture) after 18 h of incubation with ILT-156 cells, donor-derived HTLV-I-infected ILT-167 cells, and recipient-derived HTLV-I-negative LCL-156 cells at an effector cell/target cell ratio of 10. (E) The cytotoxicity of the CTLs from patient 156 (at 41 days of culture) against radiolabeled target ILT-156 cells was evaluated by 6-h ^{51}Cr release assay in the presence of the indicated unlabeled competitor cells. Both the effector cell/target cell and competitor cell/radiolabeled target cell ratios were 40:1. All IFN- γ enzyme-linked immunosorbent assay values represent the means and standard deviations from duplicates, and ^{51}Cr release assay values represent the means and standard deviations from triplicate assays.

tested had been reconstituted by that derived from donor 167, as determined by short tandem repeat (STR) polymorphism. By using similar methods, we assessed the origin of the CTLs from post-HSCT patient 156. As shown in Fig. 1C, the pattern of STRs of the CTLs was identical to that of ILT-167 cells but

not ILT-156 cells, clearly indicating that CTLs from post-HSCT patient 156 were derived from donor 167. We then examined whether the CTLs from post-HSCT patient 156 recognized potential GVH antigens expressed in ILT-156 cells but not in ILT-167 cells, besides HTLV-I. As shown in Fig. 1D, the CTLs induced from post-HSCT patient 156 equally recognized ILT-167 and ILT-156 but not LCL-156. Furthermore, cytotoxicity of the CTLs against radiolabeled ILT-156 was competed with unlabeled ILT-167 cells as well as ILT-156 cells significantly and to similar extents but was not competed with LCL-156 cells (Fig. 1E). These results indicated that CTLs from post-HSCT patient 156 were directed mainly to HTLV-I antigens commonly expressed in ILT-156 and -167 cells but not to potential GVH antigens expressed only in ILT-156 cells.

We next performed mapping analysis on the epitopes recognized by CTLs from post-HSCT patient 156 by using a panel of oligopeptides of HTLV-I Tax (6, 12), the major target antigen for HTLV-I-specific CTLs. LCL-156 cells, pulsed with a series of 15- to 24-mer oligopeptides corresponding to the amino acid sequence of the whole region of Tax, were incubated with CTLs from patient 156. Among 28 oligopeptides used, Tax81-104 (Fig. 2A) and Tax271-285 (Fig. 2B) selectively sensitized CTLs to produce IFN- γ . We then prepared five 9-mer peptides inside Tax81-104 and Tax271-285 sequences, which were predicted by computer analysis to bind HLA-A*1101 based on the anchor motifs in two databases (the BIMAS and SYFPEITHI databases) (14, 17). Among these peptides, we found that Tax88-96 (KVLTPPITH) and Tax272-280 (QSSSFIFHK) (Table 1) were dominantly recognized by the CTLs from post-HSCT patient 156.

Finally, we used phycoerythrin (PE)-conjugated HLA-A*1101/Tax88-96 and HLA-A*1101/Tax272-280 tetramers, which were prepared through the NIAID Tetramer Facility (Atlanta, GA), to directly detect HLA-A11-restricted Tax-specific CTLs. As shown in Fig. 3A, in the PBMC culture from post-HSCT patient 156 at 41 days from the initiation of culture, 5.7% of cells were positive for HLA-A*1101/Tax88-96 tetramer and CD8 and 5.8% of cells were positive for HLA-A*1101/Tax272-280 tetramer and CD8. When a mixture of both tetramers was used, 10.3% of the cells bound to these tetramers. These data clearly indicate that CTLs recognizing each epitope equally expanded in the PBMC culture derived from post-HSCT patient 156 in response to stimulation with pre-HSCT cell line ILT-156.

We further applied tetramer staining for uncultured PBMCs from post-HSCT patient 156 (Fig. 3B) and donor 167 (Fig. 3C). Although low levels of nonspecific stain were observed in the PBMCs of the seronegative donor 167, significantly higher percentages of the PBMCs of post-HSCT patient 156 were stained with CD8 MAbs and the tetramers: 0.48% for HLA-A*1101/Tax88-96 tetramer, 0.71% for HLA-A*1101/Tax272-280 tetramer, and 1.87% for both tetramers.

A number of CTL epitopes restricted by HLA-A2, -B14, and -B15 have been identified in HTLV-I Tax; most were found in the context of HTLV-I-specific CTLs derived from HTLV-I-associated myelopathy/tropical spastic paraparesis patients and asymptomatic HTLV-I carriers (2, 12, 13, 16, 25). However, to our knowledge this is the first report demonstrating HLA-A*1101-restricted Tax epitopes recognized by HTLV-I-specific CTLs. The phenotypic frequencies of HLA-A11 are 10% in

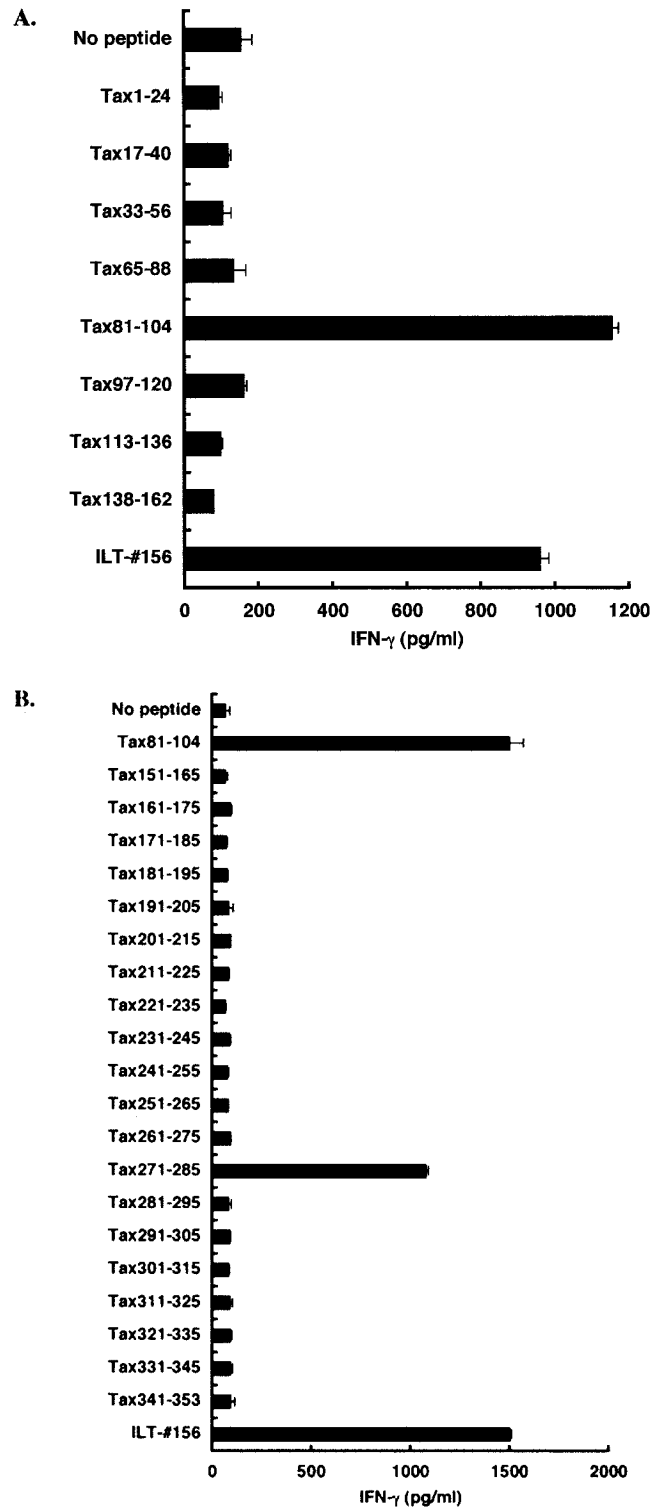


FIG. 2. Mapping of HTLV-I Tax epitopes recognized by CTLs from post-HSCT patient 156. LCL-156 cells were pulsed with 10 μ M of a series of 24-mer synthetic oligopeptides covering the N-terminal half (A) and a series of 15-mer oligopeptides covering the C-terminal half (B) of the Tax amino acid sequence, and their susceptibility to CTLs of post-HSCT patient 156 was measured by IFN- γ enzyme-linked immunosorbent assay following 18 h of incubation at an effector cell/target cell ratio of 10. Values represent the means and standard deviations from duplicate assays.

TABLE 1. Reactivity of the Tax-specific CTLs to 9-mer oligopeptides with binding motifs to HLA-A*1101 within Tax81-104 and Tax271-285^a

Peptide	Sequence	IFN- γ production (pg/ml) ^b
None		13.5 \pm 4.4
Tax81-104	Q R T S K T L K V L T P P I T H T T P N I P P S	1,080.2 \pm 92.6
Tax82-90	R T S K T L K V L	19.6 \pm 8.1
Tax88-96	K V L T P P I T H	1,201.6 \pm 55.9
Tax271-285	L Q S S S F I F H K F Q T K A	957.2 \pm 47.0
Tax270-278	V L Q S S S F I F	104.4 \pm 6.4
Tax272-280	Q S S S F I F H K	1,255.3 \pm 13.4
Tax276-284	F I F H K F Q T K	79.1 \pm 46.8

^a CTLs induced from post-HSCT patient 156 at 41 days after initiation of culture were examined for IFN- γ -producing ability by enzyme-linked immunosorbent assay against LCL-156 cells pulsed with 10 μ M of indicated Tax peptides (>80% purity) at an effector cell/target cell ratio of 10. All 9-mer peptides within Tax81-104 and Tax271-285 listed were selected based on the binding motif for HLA-A*1101 by using two databases (the BIMAS and SYFPEITHI databases) (14, 17) for HLA binding peptide prediction.

^b Values represent the means and standard deviations from duplicate assays.

Caucasians, 33% in Chinese, 20% in Japanese, and 4% in black North Americans (19, 21). The newly identified HLA-A11-restricted epitopes together with previously identified epitopes can thus be applied to a large portion of the world's population.

A major challenge in the field of allogeneic HSCT is to prevent the alloreactivity that leads to GVHD while preserving a GVL effect (4). Although it is still not clear whether Tax could be a GVL target in ATL, our present study and earlier studies (6) suggest the presence of Tax antigen presentation in

vivo in ATL patients and the potential contribution of these CTLs to GVL effects.

In summary, we identified two HLA-A*1101-restricted HTLV-I-specific CTL epitopes that were recognized by CTLs induced from an ATL patient after HSCT. The identified epitopes broaden the adaptable population for potential immunotherapy for ATL as well as for the monitoring of HTLV-I-specific CTL responses.

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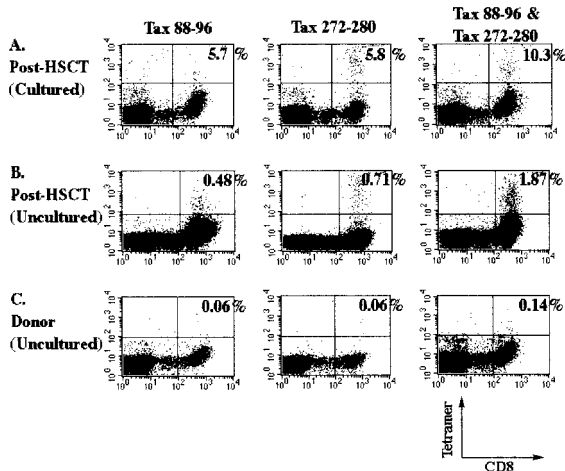


FIG. 3. Detection of Tax88-96 and Tax272-280-specific CTLs by tetramers in PBMCs from post-HSCT patient 156. CTLs from post-HSCT patient 156 at 41 days after initiation of culture (A), uncultured PBMCs from post-HSCT patient 156 (B), and uncultured PBMCs from donor 167 (C) were stained with PE-Cy5-labeled CD8 MABs (HIT8a; BD PharMingen) together with PE-conjugated HLA-A*1101/Tax88-96 (left), HLA-A*1101/Tax272-280 (center), or a mixture of both tetramers (right). Both tetramers were provided by the National Institute of Allergy and Infectious Diseases Tetramer Facility, Emory University, and were used at a dilution of 1:800. Numbers in the upper right corners indicate percentages of CD8-positive cells bound to the tetramer as analyzed on a flow cytometer (1). A total of 100,000 events were collected in each case.

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